Memorandum of Video-Conference
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Subject: Laurate Canola

Keywords:
rapeseed, *Brassica napus, Umbellularia californica*, canola, *Agrobacterium*, Laurate canola oil

Introduction:
This video conference was intended to bring Calgene's consultation with FDA, which started on August 17, 1992, to closure. Calgene had previously submitted (February 24, 1995) a summary of the safety assessment of their transgenic rapeseed (Laurate Canola: Determination of Safety and Compliance with FDA Policy). Laurate canola has been genetically engineered to produce lauric and myristic acids in the seed oil by

expression of a gene from California bay laurel (*Umbellularia californica*) that codes for the enzyme 12:0 acyl carrier protein (ACP)-thioesterase.

Introduced Genetic Material

Calgene described the identity and function of the genetic material introduced into the production organism, rapeseed (canola, (*Brassica napus* x *B. rapa* syn. *B. campestris*)) using the *Agrobacterium* transformation system. Calgene presented a summary of the Southern blot analysis, PCR analysis, and other data for two transformants to show that they have: 1) properly identified the sequences that were inserted into the plant genome, 2) ascertained that the initial transformants contained 15 copies of the introduced genetic material integrated at 5 insertion sites, and 3) the insertions did not cause any chromosomal rearrangements, although some genetic segregation is expected over time as the line is carried forward.

Identity and Function of Expression Products Encoded by Inserted Genetic Material

Two new proteins are expressed in the transgenic rapeseed. One protein that is expressed is the enzyme aminoglycoside 3'-phosphotransferase II (APH(3')II), which is encoded by the kan^r (npt II) gene originally isolated from transposon Tn 5 isolated from *E. coli.* The kan^r gene is used as a selectable marker.

The other protein expressed is the enzyme 12:0 acyl carrier protein (ACP) thioesterase (TE), which is encoded by the TE gene from California bay laurel (*Umbellularia californica*). The enzyme cleaves ACP preferentially from the naturally occurring lauroyl-ACP (12:0-ACP), and to a lesser extent from the 14:0-ACP (myristyl-ACP), during fatty acid biosynthesis. This TE enzyme function results in the preferential accumulation of the 12 carbon, saturated fatty acid, laurate, and to some degree the 14 carbon saturated fatty acid, myristate, in triacylglycerol molecules in the rapeseed oil.

Safety of the Introduced Protein

As mentioned above, the new variety contains two added proteins, namely APH (3') II and 12:0 TE. Calgene noted that the safety of this APH(3') II in the development of new varieties of rapeseed has been previously addressed (21 CFR 173.170 and 573.130).

Calgene concluded, based on a number of criteria and analyses summarized in their document, that the introduced 12:0 TE protein was not a safety concern. The 12:0 TE from *Umbellularia* is similar to long chain TE's found in canola, soybean and other edible species. The introduced TE enzyme is not detectable by Western blot analysis in mature seed, meal, or oil, and is subject to protease degradation. Finally, Calgene determined that the 12:0 TE did not meet the profile typical of a known allergen or have significant homology with known allergens.

Compositional Analysis

Toxicants and Nutrients

The intent of the genetic modification made by Calgene was to modify the fatty acid composition of canola oil. Calgene did not anticipate any other effect. However, to preclude any possibility that an unintended effect may have rendered either the meal

or oil unsafe, Calgene conducted extensive tests on the composition of both these products and compared the results to a number of control varieties.

Calgene presented the results of analyses of the naturally occurring toxicants (alkenyl glucosinolate, erucic acid) as well as the antinutritional factors (phytic acid and sinapine). Calgene concluded, based on the analytical assays used to determine the levels of the naturally occurring toxicants and antinutritional factors, that they are present at or below currently accepted values.

Calgene presented data on the nutrient profile of canola meal from the new variety compared to meal from traditional varieties. Calgene reported that the nutritional components that are significant to human and/or animal nutrition are within the accepted range for canola.

Laurate Canola Oil

The intended effect of Calgene's modification is to alter the fatty acid composition of canola oil. The oil resulting from the genetic modification (Laurate Canola oil) is a mixture of triacylglycerol molecules (triglycerides) composed of saturated and unsaturated fatty acids, and specifically containing ≤ 2% erucic acid and a range from 15 to 66 mole % laurate (see submission Table 2G). Laurate Canola oil does not meet the specification for canola oil in the Food Chemical Codex (Committee on Food Chemical Codex, 1992). The amounts of oleic acid may be significantly lower, and the amount of lauric and myristic acid are higher in Laurate canola oil than in canola oil (see submission Table 3A). Therefore, Calgene proposed and was assigned CAS No. 158318-72-0 (Canola oil, lauric acid-high) and proposed to FDA the common or usual name "Laurate Canola oil" (see submission Appendix 1).

Conclusion

Based on the safety and nutritional assessment Calgene has conducted, Calgene concluded, in essence, that the new rapeseed (canola) variety that they have developed is not materially altered in any respect relevant to food or animal feed safety from rapeseed (canola) varieties currently on the market and that it does not raise issues that would require premarket review or approval by FDA. Calgene also concluded that, based on the compositional differences of the oil and the intended use, as a replacement for lauric oils such as palm kernel oil, that a new common or usual name (Laurate Canola oil) is appropriate to distinguish this oil from Canola oil as defined by FCC. At this time, based on Calgene's description of its data and analysis, the agency considers Calgene's consultation on this product to be complete.

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